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REQUIREMENTS NECESSARY FOR CONSISTENT TRANSMISSION OF CUCUMBER MOSAIC VIRUS AND SCREENING PEPPERS (*CAPSICUM* SPP. L.) FOR CMV RESISTANCE.

A Thesis Presented

by

Paul John Pasko

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

May, 1982

Plant and Soil Sciences

REQUIREMENTS NECESSARY FOR CONSISTENT TRANSMISSION OF CUCUMBER MOSAIC VIRUS AND SCREENING PEPPERS (*CAPSICUM* SPP. L.) FOR CMV RESISTANCE.

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Approved as to style and content by:

Clark W. Nicklow, Chairperson of Committee

Gary W. Moorman, Member

George J. Hochmuth, Member

Allen V. Barker, Department Head
Plant and Soil Sciences

DEDICATION

To Mom, Dad, and

the rest of my family.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude and appreciation to his major advisor, Dr. Clark W. Nicklow, and committee members, Drs. Gary W. Moorman and George J. Hochmuth, for their advice, support, and constructive criticisms.

Sincere appreciation is also extended to the faculty, staff, and farm crew of the University of Massachusetts, Suburban Experiment Station who were always willing to give assistance whenever it was needed.

ABSTRACT

Consistently high rates of transmission of cucumber mosaic virus (CMV) and rapid symptom development in peppers (Capsicum spp. L.) were obtained when the following conditions were met: (1) temperature after inoculation was maintained at 27° or 32°C; (2) daylength was kept at 20 hours; and (3) the pepper leaf used for CMV inoculum contained CMV crystals. CMV fruit symptoms developed when pepper plants were inoculated 8 weeks after flower budding during the winter months, or 4-5 weeks after budding during the late spring and early summer. All of the pepper plant introductions screened were susceptible to CMV when mechanically inoculated. Plant introductions 288941 and 286419 exhibited high percentages of symptomless plants after aphid inoculation of CMV.

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CHAPTER I

INTRODUCTION

Cucumber mosaic virus (CMV) is a highly destructive pathogen known to attack a wide range of crops. When CMV causes disease in peppers, symptoms include stunting, leaf necrosis, chlorosis, and necrotic spotting of the fruit. The percent of fruit which are blemished increases rapidly as the virus disease progresses through the field. Such fruit are not marketable.

CMV is a serious problem in Massachusetts, inflicting severe economic losses. Some growers have been forced to make drastic cutbacks on their pepper production and others have stopped growing peppers. To date, no known resistance to CMV is available in bell peppers. Research at the University of Massachusetts, Suburban Experiment Station in Waltham has found CMV to be a difficult virus to consistently transmit. The primary objective of this research was to identify the important factors for the successful transmission of CMV so that a reliable technique could be established to consistently transmit the virus. This technique was used to screen selected pepper plant introductions for resistance to mechanical and aphid transmission of CMV.

Finally, the relationship between the stage of plant development at which inoculation occurs and the expression of symptoms on pepper fruits was determined.

CHAPTER II

LITERATURE REVIEW

Cucumber mosaic virus (CMV) causes disease in many different crops. CMV was first described by Doolittle (12) as an infectious disease which caused a mottling of both fruit and leaves of the cucumber plant. The disease was initially found to occur generally in Wisconsin, Michigan, and Indiana, and in some areas of Ohio, Iowa, Illinois, Vermont, New York, Massachusetts, and Virginia (19).

CMV is known to infect a wide range of plant species, including more than 40 dicotyledonous and monocotyledonous families (24). Under natural conditions, CMV is short-lived in dead tissue and therefore must survive in a perennial host or be seedborne to initiate new infections (15). Duffus (14) reported that of 540 plants sampled, representing over 50 species as possible field sources of five pepper viruses, 75 of the samples yielded isolates of tobacco mosaic (TMV), or cucumber mosaic viruses. Faan and Johnson (15) discovered CMV overwintering in 15 perennial hosts, of which only five were wild species; milkweed, catnip, common motherwort, flowering spurge, and white cockle. CMV transmission through seed occurs to varying degrees in 19 species, including some weeds (17). Tomlinson, et. al. (43) found 5-8% seed transmission of CMV in experimentally infected plants of chickweed (Stellaria media).

CMV is readily transmitted by mechanical inoculation with sap (17). Infection results in a variety of symptoms depending on a particular virus strain and host cultivar. The most damaging strains of the virus cause stunting, interveinal chlorosis, and an oak-leaf necrotic line pattern on the lower leaves. A faint mottling of the leaves is charac-

teristic of mild strains of CMV (24). The many symptom variants render the virus difficult to identify from symptoms alone (17). Some strains of CMV are well characterized by their infectivity on certain indicator plants. The Y strain produces local lesions on cowpea (Vigna sinensis) (37), and the spinach strain produces severe chlorosis, distortion and curling of the young leaves of tobacco (Nicotiana tabacum) (16).

CMV is transmitted in nature by more than 60 species of aphids (24). Myzus persicae and Aphis gossypii are thought to be the principal vectors transmitting the virus in a non-persistent manner (39). In laboratory experiments, Stimman and Swenson (41) found aphid transmission of CMV to be inefficient and erratic. The highest aphid transmission rates were obtained by using virus source plants which had been infected only nine days. Best results were obtained by raising the daytime temperature in the greenhouse from 24° to 32°C and the night temperature from 18° to 26°C. High temperature shortened the incubation period of the virus. Simons (39) suggested that the choice of vector species and inoculum source plant species may be important in aphid transmission experiments. He obtained better transmission of CMV using Myzus persicae than when Aphis gossypii were used and green peppers were found to be a better virus source plant than cucumbers.

Badami (3) found that a strain of CMV isolated from spinach, which had been maintained for a number of years by mechanical inoculation, lost its affinity with its usual vector aphid, M. persicae, and could no longer be transmitted by that species of aphid. He speculated that the virus itself changed, because the aphid was still able to transmit other CMV strains and the original strain was still transmissible by other aphid

species.

Gibson and Plumb (18) report that there is tremendous potential for controlling CMV by breeding aphid-resistant cultivars. However, they stress that the realization of this potential depends on understanding the interaction between the plant host, aphid, and virus.

The environmental conditions in which host plants are grown influences the symptoms produced by certain viruses. Foster and Webb (16) state that while other factors may be important, temperature has the strongest influence on virus symptom expression. They found CMV to be in a greater concentration in infected muskmelon plants grown at 29.4°C than in plants grown at 18.3°C. High temperature has also been found to influence the rate of passage of CMV through cowpea leaves (46) and to decrease the incubation period of CMV in inoculated spinach plants (8). Matthew (27) found the number of lesions on bean plants inoculated with tobacco necrosis virus to increase as the temperature increased from 55° to 82°F.

Pound and Singh (34) stressed the importance of considering temperature in evaluating breeding material for disease resistance. Working with TMV in susceptible and resistant peppers, they found the leaves of the resistant variety to be free of virus at 16°-20°C. However, at 24° and 28°C, the virus concentration in the resistant variety was higher than the virus concentration in the susceptible variety. They concluded that disease severity and virus concentration increase with increase in temperature. This work substantiated an earlier study by Pound (32) which reported that progenies of cabbage highly resistant to mosaic virus expressed resistance only at 24°C or below. At 28°C severe symptoms

developed in these plants.

Photoperiod and time of day of inoculation have been shown to influence the expression of virus symptoms. Virus concentration increases with increased photoperiod in both rape (33) and spinach (8). In squash and pumpkin, squash mosaic virus made its greatest initial increase in plants grown at high temperatures (26.5°C) under long days (20 hrs.) (4). Matthews (26) found that afternoon inoculations of tobacco necrosis virus produced more local lesions than those made very early in the morning.

CMV is relatively unstable in plant extracts, losing its infectivity if held at 60°C for 10 minutes (42). At room temperature, infectivity is lost within a few days and, in some instances, hours (17). Hidako and Tomaru (20) have achieved some success in maintaining viable CMV for extended periods in vacuum freeze-dried infected tobacco leaves.

Relying on the evaluation of the severity of the symptoms while breeding for CMV resistance enables selection only if obvious differences in symptoms exist (25). Christie and Edwardson (9) reported the presence of large virus aggregates (inclusions) in cells from plants infected with several isolates of CMV. The authors stated that since such inclusions have not been reported in other virus groups, they may possibly be used for diagnosing CMV infections. These inclusions were found most often in mesophyll cells, but were occasionally observed in the epidermis. These inclusions stained red to violet when the Azure A stain was utilized. Other diagnostic methods available for CMV include enzyme-linked immunosorbent assay (ELISA) (25) and the use of indicator plants such as Vigna sinensis, Cucumis sativus, Nicotiana glutinosa, and Chenopodium quinoa (7).

One of the major economic crops affected by CMV is pepper (Capsicum spp. L.). Virus-like symptoms were first found on peppers in the United States over 55 years ago (45). CMV has been detected in pepper fields in Texas (44), Florida (1, 2, 39, 40), Louisiana (5), Massachusetts (28), New Jersey (13), and Quebec (21). The symptoms of CMV on peppers include mottling, chlorosis, and oak-leaf line markings on the leaves and concentric yellow rings on the fruit. These rings may become necrotic and result in fruit spotting (13). Cordrey and Bergman (11) reported stunted growth and a reduction of certain elements in CMV infected pepper plants.

To date, no immunity to CMV is known in the sweet bell pepper, Capsicum annuum (31). However, some lines of C. frutescens have shown a tendency to escape infection by the virus (5). Simmons (38) reports that Tabasco (C. frutescens) is highly susceptible to CMV in the seedling stage, but becomes resistant as the plant matures. Older plants restrict the virus to single leaves and prevent the virus from being translocated throughout the plant.

Efforts to control CMV in the field by altering vector efficiency and by cultural practices have met with limited success (45). Techniques to alter the aphid vector efficiency include the use of reflective mulch (10), oil emulsion sprays (22, 23, 29), and sticky yellow polyethylene aphid traps (10). Applications of insecticides do not significantly reduce the spread of the virus because CMV is a non-persistent virus and can be readily transmitted by brief aphid probes. Due to the numerous CMV host species, eradication of overwintering hosts is impractical (35). Zitter and Simons (47) suggest using cultural practices such as the isolation of "at risk" crops from surrounding crops which may harbor both

aphid vectors and virus diseases and using non-host crops in interplantings as barriers.

Resistance to certain pepper viruses has been introduced into horticulturally acceptable peppers from suitable wild types. As the result of many years of pepper breeding, commercial pepper varieties with high levels of resistance to tobacco etch virus, potato virus Y, tobacco mosaic virus, and pepper mottle virus are now available (45).

C H A P T E R I I I

MATERIALS AND METHODS

Experiment 1 - Temperature and Daylength Studies

Pepper plants (Capsicum annuum var. 'Midway') were mechanically inoculated in the 3-4 leaf stage with sap obtained by grinding cucumber mosaic virus (CMV) infected pepper leaves in a 0.05 M potassium phosphate (Dibasic) buffer solution (pH 7.2). Nine milliliters of buffer were added to each gram of leaf tissue. The original source of virus was a diseased pepper plant growing near Andover, MA. Cotton swabs were used to apply the inoculum to carborundum (320 grit) dusted leaves. After the inoculum was applied, the leaves were rinsed with tap water. After inoculation, the plants were placed in a growth chamber (Percival, model #E-54U), and a wide range of environmental conditions were examined for their effect on CMV symptom expression. Inside the growth chamber, with plants approximately 17 inches from the light source, the light intensity at shelf level was measured at 830 foot candles.

Three different temperatures were tested, 21°, 27°, and 32°C, while the daylength was kept constant at 20 hours. Eight plants were mechanically inoculated in the 3-4 leaf stage and placed in the growth chamber with two noninoculated checks. Three days after inoculation, epidermal tissue was removed from each plant, stained with Azure A (as outlined below), and examined under the microscope for CMV crystals. The tissue was obtained from the mid-vein on the underside of an inoculated leaf. The above procedure was repeated every other day until crystals were observed in each of the eight plants. The time between inoculation and the initial appearance of crystals in the inoculated leaf was defined as the in-

cubation period. Three trials were run for each of the three temperatures.

Daylengths of 5-, 10-, and 20-hour were tested while the temperature was kept constant at 32°C. The procedure after inoculation was identical to that detailed above. Three trials were run under each daylength.

A test was conducted using aphids to inoculate eight 'Midway' pepper plants. The aphid inoculation procedure involved the physical transference of five green peach aphids, Myzus persicae (Sulzer), from a CMV infected plant to a test plant. The aphids were starved for approximately 20 hours and then allowed to probe CMV infected plants for one minute. The aphids were then transfered, using a camel hair brush, to the test plant for one hour after which time they were killed by spraying with acephate at a rate of 0.6 grams of active ingredient (a. i.) per liter. The plants were then placed in the growth chamber at 32°C and 20-hour daylength. The procedure for determining crystal development followed that which was outlined above. Three trials were run.

All plants were grown in 4-inch pots and fertilized once with Peter's 20-20-20 (N-P-K) at 100 ppm N before being placed in the growth chamber. The data were subjected to analysis of variance, and significant differences between means established by Duncan's multiple range test.

Experiment 2 - The Relationship Between CMV Crystal Size and Virus Transmission Rate

Individual leaves which had been inoculated with CMV were removed from pepper plants which were being maintained as virus source plants. The age of infection for each source plant varied. Epidermal tissue was removed from the underside of the leaf, stained using the Azure A technique (9), and observed under the light microscope. The staining proced-

ure is as follows:

A. Stain Preparation -

1. Place 18 drops of 0.1 g/100 ml. Azure A (dissolved in Ethyline glycol monomethyl ether) on a spot plate. Azure A is manufactured by Fischer Chemical Company.

2. Add 2 drops of 0.2 M NA_2HPO_4 (dibasic sodium phosphate) and stir.

B. Staining Epidermal Strips -

1. Remove epidermal strips from the mid-vein on the lower surface of the leaf. Float the tissue on the spot plate so that the torn surface is in contact with the stain (i.e., cuticle up).

2. Stain for about 15 minutes.

3. Transfer the epidermal strip to 95% ethanol and swirl for 5-10 seconds.

4. Transfer the epidermal strip to ethylene glycol monomethyl ether acetate for 10-15 minutes.

5. Mount in Euparal on slide and cover with a #1 cover slip.

6. Scan with compound microscope equipped with bright field optics (100X, oil) for crystalline inclusions.

Twenty inclusions were chosen at random and measured using an eyepiece micrometer. The twenty measurements were averaged together to obtain an average crystal size for the individual leaf. The inoculated leaf was then ground up in 0.5 M phosphate buffer (pH 7.2) and used to inoculate 10 pepper plants (var. 'Midway') in the 3-4 leaf stage which had been transplanted into 4-inch pots. After 9 days in the growth chamber (27°C, 20-hour daylength),the plants were examined for CMV symp-

toms and for the presence of viral inclusions. Nine crystal sizes were utilized in the initial trial. The experiment was repeated using nine crystal sizes which were approximately the same as those in the first trial. The data were subjected to an analysis of variance and significant differences between means established by Duncan's multiple range test.

Experiment 3 - CMV Infection Age

Ten pepper plants (var. 'Midway') were mechanically inoculated with CMV and placed in the growth chamber (27°C, 20-hour daylength). At various number of days after inoculation, inoculated leaves from these source plants were removed and examined for CMV crystals as outlined above. If the leaf contained crystals, it was used to inoculate 10 pepper plants (var. 'Midway'). After 9 days in the growth chamber (27°C, 20-hour daylength), the plants were examined for CMV symptoms and for the presence of viral inclusions. Nine infection ages were utilized in the initial trial. The experiment was repeated using infection ages approximately the same as those in the first trial. The data was subjected to an analysis of variance, and significant differences between means established by Duncan's multiple range test.

Experiment 4 - CMV Fruit Symptom Expression

Approximately one week after the initial appearance of flower buds, four pepper plants (var. 'Midway') were mechanically inoculated in the greenhouse as outlined above. A separate set of four plants was inoculated each week for a period of 12 weeks. At the time of inoculation, the number of leaves on each plant was recorded. As the experiment progressed, mature fruit was harvested and examined for CMV fruit symptoms

which include chlorosis and necrotic spotting. The experiment was terminated 4 weeks after the final inoculation. Two trials were run.

The experimental design used was that of a randomized block with two test plants and one control for each of two replicates. The data was subjected to analysis of variance, and significant differences between means established by Duncan's multiple range test. The initial experiment was begun December 15, 1980 when greenhouse temperatures ranged between 16°-21°C. When the experiment was repeated on May 13, 1981, daytime temperatures were much higher, often reaching 32°C.

Plants were grown in 11 liter plastic pots, fertilized every other week with Peter's 20-20-20 (100 ppm N), and were sprayed with acephate (0.6 gms. a.i./liter) to control aphids.

Experiment 5 - Resistance Screening

Mechanical inoculation - Thirty-two pepper plant introductions (P.I.) were obtained from the Southern Regional Plant Introduction Station, Georgia (Table 8). Fifteen plants from each P.I. were mechanically inoculated with CMV in the 3-4 leaf stage as outlined in Experiment 1. Two plants from each P.I. were maintained as noninoculated checks. Five plants from a commercial variety ('Midway') were inoculated to check the viability of the inoculum. After inoculation, the plants were placed in a controlled environment chamber (24°C, 16-hour daylength). If plants failed to exhibit CMV symptoms within two weeks, they were reinoculated. Plants surviving three inoculations were allowed to self-pollinate. The progeny from these selections were screened again as above. Plants were grown in 4-inch pots and fertilized with Peter's 20-20-20 (100 ppm N) every other week. To control aphids, acephate (0.6 gms. a.i./liter) was

sprayed every other week.

Aphid inoculation - Progeny from 12 plants representing four pepper plant introductions; P.I. 286419, P.I. 288941, P.I. 159236, and P.I. 288933 were selected to be screened for resistance to aphid-transmitted CMV. The parental plants had survived two previous aphid inoculations, performed by Dr. Gary W. Moorman and Mr. Richard A. Klemmer at the Univery of Massachusetts, Suburban Experiment Station, Waltham, MA. For each selection, 15 plants were inoculated with aphids and 3 were mechanically inoculated using the sap of the leaf on which the aphids had fed. The plants were inoculated in the 3-4 leaf stage and transplanted into 4-inch pots. Two plants of each P.I. were not inoculated to serve as checks. The plants were kept in the greenhouse where daytime temperature ranged from 24°-29°C and nighttime 18°-21°C. The plants were sprayed with acephate (0.6 gms. a.i./liter) on a weekly basis to control aphids and fertilized with Peter's 20-20-20 (100 ppm N) every other week. Plants free of CMV symptoms after 3 weeks were transplanted into larger pots and allowed to self-pollinate.

C H A P T E R I V

RESULTS

Experiment 1 - Temperature and Daylength Studies

The effect of temperature on the incubation period of CMV was evaluated by determining the percentage of 'Midway' pepper showing virus crystals 3, 5, and 7 days after inoculation at certain temperatures (Table 1). Incubation period was defined as the time between inoculation and the initial appearance of crystals in the inoculated leaf. After 3 days incubation, 46 percent of the plants grown at 32°C contained virus-induced crystals and no crystals were observed in plants grown at 27° or 21°C. After incubating 5 days, crystals were evident in all of the plants grown at 32°C and in 95 percent of the plants grown at 27°C. No crystals had yet formed at 21°C in 5 days. Seven days after inoculation, all plants at each temperature exhibited virus crystals. Mottling usually appeared at the same time crystals could be observed in the cells of the inoculated leaf. However, as infection age increased, virus crystals were not always present even though mottling was severe.

The effect of daylength on the incubation period of CMV was studied (Table 2) while the temperature was kept constant at 32°C. Three days after inoculation, 46 percent of the plants grown under a 20-hour daylength exhibited crystals, while 17 percent of the plants grown under 10 hours of daylength displayed crystals and none of the plants grown under 5 hours of daylength contained crystals. Five days after inoculation, virus crystals were found in one or more plants grown under each of the three daylengths. However, crystals in the 5-hour plants were small and sparse, and leaf symptoms were mild. One week after inoculation, CMV

Table 1. Percent plants containing CMV crystals 3, 5, and 7 days after mechanical leaf inoculation of 'Midway' pepper plants at different temperatures. Daylength constant at 20 hours.

Air Temperature (°C)	Percent plants with CMV crystals[y]		
	3 days	5 days	7 days
21	0 a[z]	0 a	100 a
27	0 a	95 b	100 a
32	46 b	100 b	100 a

[z] Mean separation within columns by Duncan's Multiple Range Test, 5% level.

[y] Cumulative data from 3 experiments. Eight plants inoculated per experiment.

Table 2. Percent plants containing CMV crystals 3, 5, 7, and 9 days after mechanical leaf inoculation of 'Midway' plants at different daylengths. Temperature constant at 32°C.

Daylength (hours)	Percent plants with CMV crystals[y]			
	3 days	5 days	7 days	9 days
5	0 a[z]	45 a	67 a	100 a
10	17 a	79 b	100 b	100 a
20	46 b	100 c	100 b	100 a

[z] Mean separation within columns by Duncan's Multiple Range Test, 5% level.

[y] Cumulative data from 3 experiments. Eight plants inoculated per experiment.

crystals were observed in all plants grown under 10- and 20-hour day-lengths. A total of 9 days was required for all the 5-hour plants to exhibit crystals.

The results of an experiment comparing the incubation periods of plants inoculated with CMV, mechanically and by aphids, is presented in Table 3. Three days after inoculation, 46 percent of the mechanically inoculated plants showed virus crystals while 21 percent of the plants which had been inoculated with aphids contained crystals. Five days after inoculation, all the mechanically inoculated plants contained crystals. Two additional days were required for crystals to develop in all of the aphid inoculated plants. No differences were observed between the two methods in the size of virus crystals, their abundancy in the cells, or external CMV leaf symptoms.

Experiment 2 - The Relationship Between CMV Crystal Size and Virus Transmission Rate

The effect of CMV crystal size on percent virus transmission was studied and the results are summarized in Table 4. When sap from a CMV infected pepper leaf containing virus crystals with an average size of 2.56 micrometers (um) was used to inoculate a set of test plants, the virus was transmitted to 95 percent of the plants. When the leaf used for inoculum contained crystals averaging 9.85 um in length, 100 percent transmission was obtained. Only one leaf, containing an average crystal size of 4.22 um, yielded a transmission rate lower than 80 percent. When no virus crystals were evident in the leaf used for inoculum, no virus transmission was obtained. Five additional inoculations, not illustrated in the table, were attempted using infected leaves with no crystals

Table 3. Percent plants containing CMV crystals 3, 5, and 7 days after inoculation of leaves of 'Midway' pepper plants inoculated mechanically or by aphids, Myzus persicae (Sulzer). Temperature and day-length constant at 32°C and 20 hours.

Days after inoculation	Percent plants with CMV crystals[y]	
	Mechanical inoculation[w]	Aphid inoculation[x]
3	46 a[z]	21 b
5	100 a	79 b
7	100 a	100 a

[z] Mean separation within rows by Duncan's Multiple Range Test, 5% level.

[y] Cumulative data from 3 experiments. Eight plants inoculated per experiment.

[x] Five aphids used to inoculate each plant.

[w] Three leaves inoculated on each plant.

Table 4. Effect of CMV crystal size on percent virus transmission in 'Midway' pepper.

Crystal size[y] (um)	Percent transmission[x]
0 (no crystals)	0 a[z]
2.56	95 d
3.76	90 cd
4.22	70 b
4.30	80 bc
4.48	100 d
5.06	95 d
7.05	100 d
9.85	100 d

[z] Mean separation within columns by Duncan's Multiple Range Test, 5% level.

[y] Average of two trials. Twenty crystal sizes averaged per trial. Size equals length of longest axis.

[x] Average of two trials. Ten plants inoculated per trial.

and no higher than 20 percent transmission of the virus was obtained.

All CMV induced crystals were located exclusively in the epidermal cells. Most of the crystals observed were angular in structure and very often aggregated together.

Experiment 3 - CMV Infection Age

The effect of age of CMV infection in the leaf used for inoculum on percent virus transmission was investigated (Table 5). Infection ages in the source plants ranged from 5 to 46 days, and each leaf used for inoculum contained virus crystals. High percent transmission was obtained from both young and old infection ages. Only one infection age (14 days) produced a percent transmission rate lower than 80 percent. At the oldest infection age (46 days), 90 percent of the plants became diseased. One trial (not illustrated) was attempted using leaves from a 45 day old infection which did not contain virus crystals and no virus transmission was observed.

Experiment 4 - CMV Fruit Symptom Expression

An experiment was conducted in the greenhouse to determine whether a relationship existed between stage of plant development at the time of inoculation and the expression of CMV fruit symptoms in 'Midway' pepper. Two separate trials were conducted. The first trial was begun December 20, 1980, while the second was started May 13, 1981.

In the initial experiment (Table 6), the majority of fruit with CMV symptoms was harvested from plants which had been inoculated 5-8 weeks after the appearance of flower buds. The highest percentage of fruit exhibiting CMV symptoms was harvested from the plants inoculated 8 weeks after budding. No fruit with CMV symptoms was harvested after inocula-

Table 5. Effect of age of CMV infection in the inoculated leaf of the virus source plant on percent virus transmission in 'Midway' pepper.

Days after inoculation of source plant[w]	Percent transmission[x]
5.0[y]	100 a[z]
7.0	100 a
10.0	100 a
12.5	80 bc
13.0	95 a
14.0	70 c
23.0	90 ab
31.0	100 a
46.0	90 ab

[z] Mean separation by Duncan's Multiple Range Test, 5% level.

[y] Each virus source plant contained CMV crystals and was maintained at 27°C and 20-hour daylength.

[x] Average of two trials. Ten plants inoculated per trial.

[w] Average of two infection ages.

Table 6. Effect of stage of plant development at time of inoculation on the percent of 'Midway' pepper fruit showing CMV symptons. Also given are average number of leaves at inoculation. Trial 1, begun 12/30/80.

Inoculation week	Ave. no. leaves at inoculation	Percent fruit showing symptoms	
		Inoculated[x]	Noninoculated[w]
1[y]	13.8	0 a[z]	0
2	23.5	0 a	0
3	28.2	0 a	0
4	41.4	0 a	0
5	42.2	10 ab	0
6	45.3	15 ab	0
7	47.0	6 ab	0
8	51.4	18 b	0
9	56.0	0 a	0
10	59.3	0 a	0
11	62.7	0 a	0
12	68.2	3 ab	0

[z] Mean separation within columns by Duncan's Multiple Range Test, 5% level.

[y] Initial inoculation done one week after 50% of plants showed flower buds.

[x] Four plants inoculated per week.

[w] Two plants noninoculated per week.

tion weeks 1-4 and only 3 percent with symptoms was harvested after the eighth inoculation week. The fruit harvested from the noninoculated plants showed no CMV symptoms.

The CMV symptoms observed on the fruit included a slight chlorosis and the presence of brown necrotic spots similar to those observed in commercial peppers infected with CMV.

In the second trial (Table 7), fruit with CMV symptoms was harvested only from the plants inoculated 3-6 weeks after the onset of flower budding. The highest percentage of fruit with CMV symptoms was harvested from the plants inoculated 4 and 5 weeks after budding. None of the fruit harvested after the sixth inoculation week exhibited symptoms. Fruit harvested from the noninoculated plants was free of symptoms. The number of leaves present at inoculation, for the weeks where CMV fruit symptom expression was optimum, ranged from 42.2 to 51.4 in Trial 1, and 55.5 to 76.7 in Trial 2.

The severity of CMV symptoms varied with the plant growth stage at which they were inoculated. In the early weeks of the experiment (i.e., weeks 1-4 in Trial 1 and weeks 1-2 in Trial 2), leaf mottling and severe stunting was evident. The fruit from these plants, while free of symptoms, never fully developed and remained small and distorted. During the intermediate weeks, when fruit symptoms began to appear, leaf mottling and stunting was slight to moderate. During the final weeks of the experiment, mottling was barely noticeable and confined to the inoculated leaf. No stunting of plant growth was observed and only 3 percent of the fruit harvested exhibited CMV symptoms. Thus, the longer inoculation was delayed after flower bud formation, the less severe were the

Table 7. Effect of stage of plant development at time of inoculation on the percent of 'Midway' pepper fruit showing CMV symptoms. Also given are the average number of leaves at inoculation. Trial 2, begun 5/13/81.

Inoculation week	Ave. no. leaves at inoculation	Percent fruit showing symptoms	
		Inoculated[x]	Noninoculated[w]
1[y]	29.0	o a[z]	0
2	38.6	0 a	0
3	55.5	12 bc	0
4	60.6	19 c	0
5	61.0	19 c	0
6	76.7	6 ab	0
7	81.3	0 a	0
8	87.5	0 a	0
9	93.0	0 a	0
10	94.6	0 a	0
11	97.1	0 a	0
12	98.0	0 a	0

[z] Mean separation within columns by Duncan's Multiple Range Test, 5% level.

[y] Initial inoculation done one week after 50% of plants showed flower buds.

[x] Four plants inoculated per week.

[w] Two plants noninoculated per week.

leaf symptoms that developed.

Experiment 5 - Resistance Screening

Mechanical inoculation - After four mechanical inoculations, none of the pepper plant introductions exhibited resistance to CMV (Table 8). Plants showing no virus symptoms after three inoculations were later determined, by screening the progeny, to be escapes. The symptoms observed included leaf mottling and stunting. Symptoms became evident approximately five days after inoculation.

Aphid inoculation - Progeny from twelve individual plants, representing four pepper plant introductions, were selected to be screened for resistance to aphid-transmitted CMV. The parental plants had survived two aphid inoculations. The number of symptomless selections from each plant introduction was as follows: P.I. 286419 - 6 plants; P.I. 159236 - 1 plant; P.I. 288933 - 2 plants; and P.I. 288941 - 3 plants. The seed from each selection was sown and the resulting seedlings inoculated with CMV using viruliferous aphids. The average percentage of plants surviving the inoculation for each P.I. is illustrated in Table 8. Plant introduction 288941 had the highest percentage (40%) of plants exhibiting no CMV symptoms after the aphid inoculation. The symptoms observed on the aphid inoculated plants were mottling and stunting similar to those observed in the mechanically inoculated plants. However, symptoms required longer to develop on the aphid inoculated plants than on those which were mechanically inoculated.

Table 8. Plant introductions of Capsicum spp. screened for resistance to cucumber mosaic virus.

P.I. no.[z]	Species	Percent plants surviving inoculation[w] Mechanical inoculation[y]	Aphid inoculation[x]
109252	C. annuum	0	-
159236	C. annuum	0	13
159261	C. annuum	0	-
159266	C. annuum	0	-
163201	C. annuum	0	-
169122	C. annuum	0	-
169133	C. annuum	0	-
169134	C. annuum	0	-
171553	C. annuum	0	-
174810	C. annuum	0	-
175622	C. annuum	0	-
178847	C. annuum	0	-
183441	C. annuum	0	-
224418	C. annuum	0	-
224419	C. annuum	0	-
224421	C. annuum	0	-
224439	C. annuum	0	-
246123	C. annuum	0	-
244668	C. annuum	0	-
241650	C. annuum	0	-

- continued -

Table 8. Continued

P.I. no.[z]	Species	Percent plants surviving inoculation[w] Mechanical inoculation[y]	Aphid inoculation[x]
257047	C. annuum	0	-
262905	C. annuum	0	-
263106	C. annuum	0	-
281416	C. annuum	0	-
286419	C. annuum	0	31
288933	C. annuum	0	16
288941	C. annuum	0	40
338947	C. annuum	0	-
257071	C. frutescens	0	-
381155	C. frutescens	0	-
391564	C. frutescens	0	-
152225	C. chinense	0	-

[z] Plant introductions obtained from the Southern Regional Plant Introduction Station, Georgia.

[y] Percent plants surviving after four mechanical inoculations. Fifteen plants per P.I. inoculated with two noninoculated controls.

[x] Percent plants surviving after three aphid inoculations. Fifteen plants per P.I. inoculated with two noninoculated controls.

[w] Plants not exhibiting CMV symptoms after inoculation were considered to have survived the inoculation.

CHAPTER V

DISCUSSION

The experiments conducted in the growth chamber emphasize the importance of considering environmental factors when working with CMV. The incubation period of CMV was found to decrease as the temperature was increased (Table 1). This supports the work of Stimman and Swenson (41) which showed the incubation period of CMV in lima beans decreased when the temperature was raised from 24° to 32°C. At the highest temperature, they reported an incubation period of 4 days. In the present study, 3 days of incubating at 32°C were required for virus crystals to appear. If a large number of plants must be screened, and limited space is available, the higher temperature and shortened incubation period speeds the screening process. However, caution should be exercised because resistance to certain viruses may be lost at certain temperatures. Pound (32) reported the loss of resistance to turnip virus 1 in cabbage at 28°C.

Increasing daylength decreases the incubation period of CMV (Table 2). This disagrees with Bancroft (4) who found no reduction in the incubation period of squash mosaic virus (SMV) in squash plants when the daylength was lowered from 20 to 8 hours. However, Pound and Garces-Orejuela (33) reported that the multiplication of turnip mosaic virus in rape is favored by long photoperiods.

Only the direct effects of daylength and temperature were investigated in this study. Other factors, such as light intensity have been shown to affect the behavior of certain viruses (4). There may also be interactions occurring between these environmental factors. Future studies are needed to investigate these possibilities.

In an experiment comparing the incubation periods of pepper plants inoculated mechanically and by aphids, a significantly higher percentage of mechanically inoculated plants contained crystals 3 days after inoculation than those which were inoculated by aphids (Table 3). Aphid transmission of some strains of CMV has been shown to be generally inefficient and erratic (40). However, in the present study, high rates of aphid transmission were obtained when the plants were kept at temperatures between 27° and 32°C and the virus source plant contained virus crystals. These conditions are similar to those shown to be necessary to obtain high rates of virus transmission by mechanical inoculation in the present study.

The presence of virus crystals was found to be a good diagnostic tool in determining whether CMV had infected the plant. This was important in the screening process since some of the plant introductions did not exhibit distinct CMV symptoms after inoculation. These virus crystals, which were readily observed under the light microscope, were measured to be approximately 2 to 10 um at their longest axis. Crystal size had little effect on percent virus transmission. Consistently high rates of transmission were obtained for both large and small crystals (Table 4). However when no crystals were present in the leaf used for inoculum, virus transmission was less than 20 percent even though the leaf exhibited distinct mottling, typical of viral disease symptoms. The presence of these crystals may be an indication of high virus titer.

High percent transmission of CMV was obtained from virus source plants with infection ages as high as 46 days (Table 5) when the leaf used for inoculum contained crystals. This contradicts Simons (40) who

reported that percent transmission of a certain strain of CMV decreases to less than 10 percent two weeks after inoculation. In the present study, the lowest transmission rate obtained (70%) was from a virus source plant with an infection age of 14 days. This low transmission rate amidst much higher rates of transmission could be a sign of cycles of high and low rates of replication of the virus. Cheo and Pound (8) have shown that a cycle of infectious virus occurs in spinach plants inoculated with CMV.

CMV has been reported to be a difficult virus to transmit both mechanically (40) and by aphids (41). Usually no better than 50 percent transmission is typical. The results of this study emphasize the value of using leaves containing virus crystals as the source of inoculum. Using this technique, consistently high rates of virus transmission are obtained. In the screening process, making sure the leaf used for inoculum contains crystals can significantly reduce the number of escape plants present after inoculation, and save a considerable amount of time by reducing the number of reinoculations needed.

The results of the CMV fruit symptom experiment suggest that the stage of plant development at the time of CMV inoculation influences symptom expression. No CMV fruit symptoms were obtained when plants were inoculated at an early or late stage of development. Simmons (38) reports that in Tabasco pepper (Capsicum frutescens) the younger the plant is at inoculation, the more severe are the CMV leaf symptoms. When older plants are inoculated, systemic spread of the virus is absent because the infected branch wilts and dies. She states that with increasing age, the multiplication and movement of the virus through the plant appears to

be hindered. These observations are similar to those of Samuel (36) who, while investigating the rate of tobacco mosaic virus (TMV) passage through tomato plants, found the virus well distributed in the plant soon after inoculation. The virus was sparsely distributed in medium age plants until approximately 3 weeks after inoculation. In old plants, 2 months were required before the virus was distributed throughout the plant. The effect of age on the movement of virus through the plant would explain why no CMV fruit symptoms were found on any of the fruit harvested from the plants which were inoculated at a later stage of development. However, the reason why the youngest plants inoculated failed to exhibit fruit symptoms, even though leaf mottling was severe, is unclear. The initial rapid rate of virus movement through the plant may have been followed by a sharp decrease in translocation. This would result in the lack of systemic spread of the virus into the developing fruit.

If CMV fruit symptoms are to be observed in the greenhouse, the plants should not be inoculated too early or too late in their development. There is an intermediate stage of development when, if a plant is inoculated, fruit symptoms will develop. This intermediate stage was reached earlier by the plants of Trial 2 (Table 7) than those of Trial 1 (Table 6), possibly due to the better growing conditions prevailing during the late spring and early summer. If CMV fruit symptoms are desired, plants should be inoculated once a week for five weeks beginning four weeks after observing the first flower buds.

The average number of leaves present at inoculation varied between trials. In the first trial, the average number of leaves present during

the eleventh inoculation week was 62.7 and no fruit with CMV symptoms was harvested from those plants. In the second trial a leaf count of 61.0 corresponds with the fifth inoculation week from which a significant amount of fruit with CMV symptoms was later harvested. Therefore, leaf count is unreliable in predicting whether fruit symptoms will develop. This CMV fruit symptom experiment was conducted under greenhouse conditions. Further study is needed to determine whether similar results can be obtained under field conditions. If peak periods of fruit symptom development can be identified in field-grown peppers, oil spray programs could be timed to protect the plants during these periods.

A high percentage of plants within two pepper plant introductions, P.I. 288941 and P.I. 286419, failed to exhibit symptoms after being aphid inoculated with CMV. If resistance is involved, the exact mechanism has yet to be determined. Pitrat and Lecog (30) have found that resistance to CMV transmission by aphids in muskmelon (Cucumis melo L.) is controlled by one dominant gene that appears to be associated with non-preference for these plants by the aphids. Non-preference controlled by a dominant gene is probably not involved in the present study because higher percentages without symptoms would be expected than were actually obtained here.

Tolerance is another possible mechanism of resistance to virus transmission by aphids (18). Pitrat and Lecog (30) noted two aspects of tolerance to CMV symptoms in certain lines of muskmelon. These were the absence of leaf curling, controlled by one dominant gene, and resistance to stunting, which appears to have a more complex inheritance. It is possible that the plants which did not become diseased in this study

merely did not exhibit the characteristic symptoms. No virus crystals were found in these plants, however this does not completely rule out the presence of virus. Other possible resistance mechanisms which may be involved (30) include aphid probing behavior which prevents CMV from reaching sites from which the virus spreads in the plant or a reaction of the plant to the aphid probes, such as a release of chemicals by the plant, which hinders the spread of the virus from cell to cell.

Plant introductions 288941 and 286419 are both classified as Capsicum annuum as is the sweet bell pepper. However, the fruit of these lines bear little resemblance to the shape, length, and lack of pungency of bell peppers. The fruit of both P.I.'s were long, slender, and highly pungent. It is not known whether the plants which did not exhibit symptoms in this study are resistant to aphid-transmitted CMV or were merely escapes. In future research, the progeny from these plants will be put through the screening process to determine whether a form of resistance does exist and to study the inheritance of the resistance. If resistance can be established, it may be possible to incorporate it into a commercial pepper line through backcross breeding.

The results of this research indicate that high transmission of CMV and rapid symptom development in peppers may be obtained if the following conditions are met: (1) temperature should be kept between 27° - 32°C; (2) daylength should be approximately 20 hours; and (3) the leaf used for inoculum must contain CMV crystals. If CMV fruit symptoms are to be obtained in the greenhouse, plants should be inoculated once a week for a period of 5 weeks beginning 4 weeks after the first flower buds are observed.

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